

Chapter 2

IN SITU ANALYSIS OF SOIL COMPONENTS
OF BIOLOGICAL ORIGIN

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I. INTRODUCTION

Some 30% of the chapters in this series have been concerned with the physical or chemical characterization of the various classes of soil organic compounds. With few exceptions, the work has been carried out on material extracted from the soil. This approach has provided

quantitative data, such as the total amount of carbohydrate in a soil and the relative proportions of individual monosaccharides. It has however, left unanswered such questions as the reasons for the apparent resistance of soil polysaccharides to microbial degradation. A similar situation exists for soil N. Only 1 to 3% of the soil N is mineralized during a season, even though nitrogenous compounds similar to those known to occur in plants and microbial products are mineralized rapidly when added to soil (Bremner, 1967). A variety of explanations have been proposed for the stability of organic matter (Clark and Paul, 1970, Cheshire, 1977) but a definite answer will require knowledge of the precise location of the organic matter in the soil fabric and its association with clays, metal ions, and other organic components.

The necessity for in situ studies of soil organic matter has been evident for some time but the acquisition of information has been restricted by the lack of suitable techniques. The application of electron optical techniques to the study of soil samples from natural systems, with their native structure intact, is beginning to provide the required information. By using electron probe microanalysis (EPM), some individual elements can be localized with high precision in soil sections and their concentrations measured. The use of scanning electron microscopy (SEM) has provided information on the morphology of soil components and on their distribution at surfaces within the soil fabric. Biological material such as microbial cells or aggregates of organic matter can be identified in ultrathin sections of resin-impregnated soil by transmission electron microscopy (TEM) in both the conventional (CTEM) and scanning (STEM) modes.

We will discuss the techniques presently available for in situ studies of soil components of biological origin with special emphasis on TEM investigations, and will present selected data to illustrate their applications, limitations, and possible future developments in this field. We will not, however, review results obtained by these techniques in studies of particular topics such as the estimation of biomass or plant production.

II. LOCALIZATION IN SOILS

A. General

Schlesinger (1977) has estimated that on a global basis there is more organic C in the detritus in and on the surface of soils (1456×10^9 tonnes C) than in the living biomass above and within it (826×10^9 tonnes C). Although the total amount of organic C at various depths is known for a variety of ecosystems and the major chemical components have been broadly and in some cases minutely characterized, detailed analysis of the morphology and spatial distribution of organic matter within soil fabrics has

remained largely neglected, even at the light microscope level. Brewer (1964), Bal (1973), and Babel (1975) have introduced systems of classification of soil organics, based mainly on morphological criteria.

Investigations have been made of the distribution of organic matter in sections of soil, taken from increasing depths in particular soil profiles, and changes have been related to pedological processes and to the action of soil fauna (Babel, 1971; Bal, 1973). Bullock (1974) and Babel (1975) have reviewed recent work on the distribution of organics in soil fabrics as determined by light microscopy. Such investigations are limited by the resolution of the light microscope and by the thickness of soil sections to particles greater than 2 μm . Electron microscopy is necessary for the in situ analysis of organic matter of submicrometer size, and, in favorable preparations, organic particles down to macromolecular sizes (e.g., 5 nm) have been observed in situ in natural soil fabrics.

The preparation for electron microscopy of soil fabrics in which all the soil components are preserved in situ is beset with peculiarly difficult technical problems. These problems arise from the physical and chemical heterogeneity of most soils at the ultrastructural level. A typical soil fabric may contain extremely hard materials such as quartz, which can only be sectioned with difficulty, and soft inorganic and organic colloids. Sand grains 1 mm or more in diameter may lie next to fragments of lipoprotein membrane only 10 nm thick. These gross differences in texture may induce compression artifacts, as well as tearing and fragmentation of ultrathin sections.

Soil components differ markedly in their stability. Most minerals are unaffected by the processes involved in preparation for electron microscopy and in the electron beam, but some clays shrink, distort, and even collapse completely on dehydration (Smart, 1974). Some organic components, such as low-molecular-weight lipids, carbohydrates, and polyphenols, may be soluble in the reagents used in fixation, dehydration, and embedment and may be removed from the fabric altogether, or redeposited at new sites. Most organics are rapidly denatured, and even destroyed, in the electron beam so that it is necessary that they are chemically stabilized and fixed in situ in the soil fabric, before examination by electron optical methods. Many soils have a loose, open texture, and mechanical support must be provided for the fabric to prevent its collapse during preparation for electron microscopy.

Whereas most of the inorganic components have high intrinsic electron density and are readily detectable by electron optical techniques, most soil organics are largely composed of elements of low atomic number (H, O, C, N) which are not at present detected routinely in electron microscopes. Some old organic materials may become naturally impregnated with elements such as Fe, Al, or Mn, but unstained, freshly deposited organics can only be seen electron-optically as transparent regions in otherwise dense fabrics. Examples are the carbohydrate

capsules surrounding bacteria in Figs. 1 and 2. In SEM, smaller fragments of organics are recognized as amorphous or fibrillar materials, coating otherwise sharply delineated mineral crystals (McKyes et al., 1974), while larger deposits can be identified by their morphology. Precise localization and positive identification of soil organic matter depends on the use of electron-dense, heavy metal compounds which are infiltrated into or deposited upon organic structures (Foster and Marks, 1967; Smart 1974; Foster, 1978). In general, those organics which do not react with metals will not be seen.

The peculiar features of soil fabrics require that a mixture of conventional methods (Pease, 1964; Kay, 1967; Pearse, 1972; Glauert, 1974) and special techniques (Smart, 1974; Tovey, 1974; Foster and Rovira, 1976, 1978) are used to prepare soil fabrics for electron microscopy. Most soils contain biological organisms such as fungi and bacteria, which have a well-known ultrastructure, and the quality of the preservation of their internal membranes provides a useful check of the adequacy of the preparation techniques. Unfortunately, these techniques limit the resolution obtainable by electron optical methods in *in situ* studies of soil fabrics. Despite high intrinsic electron density and stability, the ultrastructure of minerals *in situ* in soil sections cannot be seen with the same high resolution as when the minerals are isolated from the fabric and examined directly. In sections, details are masked by the organic embedding medium which encloses the particles. Resolution of organic materials is further reduced by the inherent granularity of the metals deposited in and on them to obtain contrast. Even so, macromolecules such as fibrils in bacterial capsules can be seen *in situ* and the electron optical methods can provide a link between the studies of the soil micromorphologist and the soil biochemist.

Full *in situ* analysis of organic matter in natural soil fabrics depends on the solution of the technical problems outlined above, in particular the following:

1. Stabilization of the soil fabric to prevent relative movement of its components during preparation for electron microscopy
2. Detection of the organic matter, including the capacity to distinguish it from finely divided or amorphous colloidal inorganic materials
3. Differentiation, both morphologically and chemically, between the different kinds of organic matter
4. Estimation of the relative proportions of the various components of the soil fabric

Of the three electron-optical methods commonly used (TEM, SEM, and EPM), only TEM of ultrathin sections of soil fabrics treated with substrate-specific stains provides information at high resolution on both the

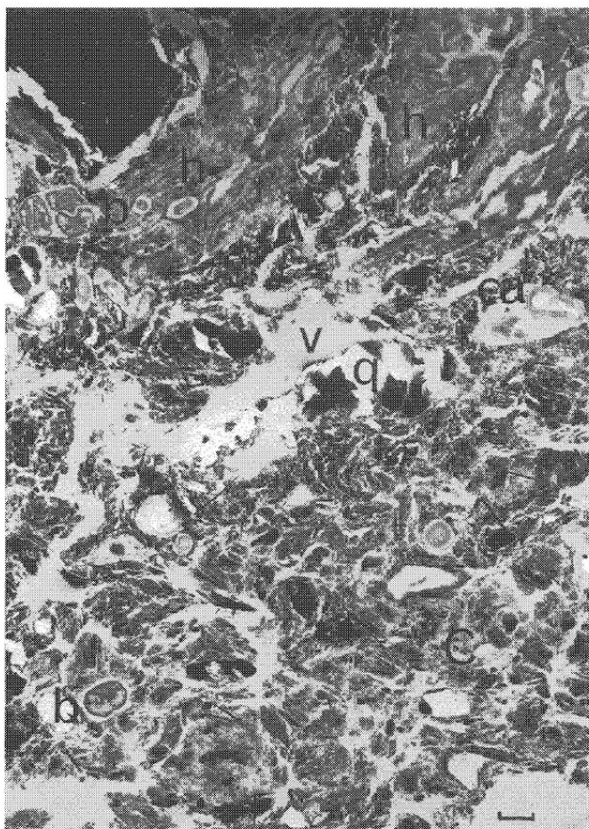


FIG. 1 Soil fabric remote from roots, showing soil bacteria enclosed in clay minerals or associated with humified organics. The bacterial capsule is unstained. Os/U/Pb. (Note: All figures in this chapter are transmission electron micrographs of ultrathin sections of natural soil fabrics and are not embedded in agar. The bar = 1 μ m. Abbreviations: b = bacteria; c = clay minerals; ca = capsule material; d = cytoplasmic debris; h = humified material; o = organic matter; q = quartz; R = compressed wall remnants; s = silver-reactive carbohydrate; v = natural void; w = cell wall.)



FIG. 2 Root-soil interface showing major, plant-derived organic materials found in soils: mucilage (m); cell walls; and bacterial metabolites [capsule, polyhydroxybutyrate (white), and polyphosphate (black granules) in bacteria]. Note lysis (l) of mucigel by bacteria. Os/La.

location and the chemical nature of soil organics. The following discussion therefore deals largely with processes necessary for the preparation of ultrathin sections of soils.

B. Methods

1. Stabilization of the Fabric

Soil fabrics may be stabilized physically by enclosing them in an organic gel (primary embedment) and chemically by treatment with organic aldehydes (primary fixation). Primary embedment with agar or gelatin has been widely used, especially by microbiologists (Hawker et al., 1962; Bullock, 1974). The combination of primary embedment in agar and primary fixation by glutaraldehyde and acrolein or formalin has been used for TEM studies of peat (Dart et al., 1969) and more normal soils (Foster, 1978). Dart et al., (1969) chemically fixed the soil fabric with aldehyde vapors before agar infiltration. Foster used a mixture of agar and aldehydes. Enhanced infiltration of the agar-aldehyde mixture was obtained by maintaining the soil at 38°C for 30 min before cooling and further fixation at 4°C (Foster, 1978). Fixation of organic materials in soils is improved if the agar-impregnated blocks are reduced to 1 cm³ for the first hour of fixation and to 1 mm³ thereafter. Preservation of biological structures may be enhanced by the addition of divalent ions such as Ca or Mg to the fixative. Gel infiltration and fixation may be performed on exposed soil profiles in the field.

It is necessary that the primary embedding medium have an amorphous, fine-grained structure at the ultrastructural level and that it does not react chemically with soil components or with the secondary fixatives or stains. Davies et al., (1964) showed that gelatin reacts with soil polyphenols and that these reactions are pH-dependent. Such reactions may interfere with subsequent staining procedures. Some humic materials are sensitive to pH changes and it is necessary to buffer the fixative. The type and pH of the buffer are limited by the nature of any subsequent histochemical reactions, e.g., tests for specific enzymes in the soil fabric (Sec. III.D.2).

2. Enhancement of the Electron Density of Soil Organics

Positive staining of organic matter may be obtained by treating the bulk, agar-embedded specimens (block staining) and/or the plastic-embedded sections (section staining) with solutions of heavy metals. Some biochemicals which do not react with heavy metals in the native state may be rendered reactive by chemical pretreatment. Four or more stages may be employed in succession as follows:

1. Treatment of the bulk, agar-embedded soil sample with substrates, specific for particular soil organics, such as enzymes
2. Treatment of the bulk specimens with nonspecific secondary fixatives containing the salts of heavy metals, such as osmium, manganese, or uranium
3. Treatment of the ultrathin, plastic-embedded sections with substrate-specific stains
4. Further enhancement of electron density of organics with nonspecific stains applied to the ultrathin sections

For routine purposes, stages 1 and 3 are omitted.

a. General heavy metal secondary fixation The most commonly used secondary fixative is OsO_4 . KMnO_4 was once widely used for biological materials, but it is now known to destroy some cellular components. The reaction of these fixatives with certain organic materials has been investigated by Bahr (1954), Hake (1965), and Bland et al., (1971). The general conclusions are summarized in Table 1.

Of the materials listed in Table 1, carbohydrate plays the major role in maintaining soil structure. Unfortunately, carbohydrate does not react with aldehydes, OsO_4 , or KMnO_4 under the conditions normally employed in biological preparations (Bahr, 1954). Proteins, lipids, and nucleic acids, which do react, are only minor constituents of the bulk soil, but they may be important components of plant exudates. A large part of the plant-derived organic matter which enters soils consists of cell wall

TABLE 1 The Reaction of Commonly Used Fixatives with Organic Materials^a

	Aldehydes	OsO_4	KMnO_4
Nucleic acids	-	-	D
Proteins	+	+	++
Phospholipids	-	++	++
Unsaturated fats	-	++	+
Polysaccharides	-	-	-

^aKey: - weak or no reaction; + moderate reaction; ++ strong reaction; D material destroyed.

Source: Adapted from Juniper et al., (1970).

TABLE 2 The Reaction of Commonly Used Fixatives With Wood Derivatives^a

	OsO ₄	KMnO ₄
<u>Gymnosperm Wood</u>		
Wood meal	+	+
Milled wood	++	+++
Milled wood lignin	++	++++
Methanol lignin	++	++++
Holocellulose	-	-
<u>Angiosperm Wood</u>		
Wood meal	++	++
Milled wood	++	++
Milled wood lignin	++	++++
Methanol lignin	-	+++
Holocellulose	-	-

^aKey: - no reaction; + weak to ++++ strong reaction.

Source: Adapted from Bland et al. (1971).

materials from stems and roots. The reaction of the more biologically stable components of these tissues with OsO₄ and KMnO₄ is presented in Table 2.

The main OsO₄- and KMnO₄-reactive materials are lignified tissues and those containing other polyphenolics. OsO₄, which is preferred by biologists, does not react so strongly with lignin as KMnO₄, and the latter may be preferable in studies of soil sections.

Humified materials contain both phenolics and amino acids (Ladd and Butler, 1971; Flaig et al., 1975) and these react with OsO₄ and KMnO₄, as shown in Table 3. The most reactive compounds are those containing di- and trihydroxy phenolic groups, unsaturated acidic, alcoholic, or aldehydic groups, and phenolic syringyl groups. Sulfhydryl and aromatic groups are the most reactive moieties of amino acids. Thus most of the electron density observed in the nonliving organic components of soils, following conventional fixation is due to polyphenolic and amino acid groups (Foster, 1978).

b. Nonspecific staining The electron density of organic materials in ultrathin sections of soil fabrics may be enhanced further by treatment with alkaline lead citrate (Reynolds, 1963), phosphotungstic acid (Hall et

TABLE 3 Reaction of Commonly Used Secondary Fixatives with Model Compounds Related to those Found in Humic Materials^a

Compound	Fixative		Compound	Fixative	
	OsO ₄	KMnO ₄		OsO ₄	KMnO ₄
<u>Phenolics</u>			<u>(Phenolics, cont.)</u>		
Phenol	+	+++	Syringin	+++++	
Catechol	+++++	+++++	Quercetin	+++++	++++
Resorcinol	+++++	+++++	<u>Amino Acids</u>		
Hydroquinone	+++++	+++++	Glycine	-	++
Pyrogallol	+++++	+++++	DL-alanine	-	-
Phloroglucinol	++++	+++++	DL-serine	-	++
Benzaldehyde	-	++	L-cystine	+	-
p-Benzaldehyde	-	+++	L-cysteine	+++++	++++
Protocatechualdehyde	+++++	++++	DL-threonine	-	+
Vanillin	+++++	+++++	DL-valine	-	-
Syringaldehyde	++	+++	DL-methionine	+++++	-
Cinnamaldehyde	+++	+++	L-leucine	-	-
Benzoic acid	-	-	L-isoleucine	-	-
p-Hydroxybenzoic acid	-	++	DL-phenylalanine	-	-
Protocatechuic acid	+++++	++++	L-tyrosine	-	++
Gallic acid	+++++	+++++	L-proline	-	++
Vanillic acid	-	+++	DL-tryptophan	++	++
Syringic acid	+++	+++	DL-aspartate	-	-
Cinnamic acid	+++	+++	L-glutamate	-	-
p-Coumaric acid	++++	+++	L-arginine	+	+
Ferulic acid	++++	+++	L-lysine	-	++
5-Hydroxyferulic acid	+++++	+++++	<u>Fatty Acids</u>		
Sinapic acid	++++	+++	Palmitic acid	-	-
Caffeic acid	+++++	+++++	Abietic acid	-	+
Ellagic acid	++++	+++	Oleic acid	++++	-
Coniferin	+++++	+++	Linolenic acid	+++++	-

^aKey: - no reaction; + slight to ++++++ intense reaction.

Source: Modified from Bland et al., (1971).

al., 1945), and/or uranyl acetate solutions (Watson, 1958). The methods are described in the standard manuals (Meek, 1970; Juniper et al., 1970; Mercer and Birbeck, 1972; Pearse, 1972). Clowes and Juniper (1968) have pointed out that the precise nature of the reaction of these stains with

particular organic groups is unknown; the action of some stains is controversial (Pease, 1975), and others may enhance the electron density of amorphous inorganic colloids. They may merely increase the electron density already conferred by the secondary fixatives. When solutions of lead salts are added to natural soils, Pb is adsorbed primarily onto the humic materials (Zimdahl and Hassett, 1977); it also reacts with some carbohydrates, clay minerals, and oxides of manganese and iron (Sec. III.D.1). General heavy metal stains, such as lead, may therefore aid detection of finely divided deposits of soil components, but may not necessarily provide information about their chemical nature.

c. Specific histochemical reactions The use of specific histochemical stains for the identification and localization, at the ultrastructural level, of organics in situ in soil fabrics has been limited by the lack of information on the precise nature of the chemical groups present in unmodified soil organic matter. Most of the published work has been about the identity of organics extracted from the fabric and degraded by enzymes (Ladd and Butler, 1971) or by other, sometimes drastic, chemical methods (Flaig et al., 1975). Specific groups present on these breakdown products may or may not be present in the unmodified soil.

Within the last ten years, methods have been introduced for the precise identification and localization, at the ultrastructural level, of a wide range of biochemicals. Their use has been largely limited to biomedical tissues, but some methods have been adapted to the kinds of plant materials likely to be found in soils. Tests for carbohydrates and proteins are of particular relevance to studies of soil fabrics, especially now that methods are available by which particular types of carbohydrate and particular proteins, such as enzymes, may be located. For example, the ultrastructure of acidic polysaccharides on roots has been investigated by the use of colloidal iron (Roland and Vian, 1970).

Mucopolysaccharides may be demonstrated by the use of a ruthenium red-OsO₄ complex (Luft, 1971). Ru Os has been used to introduce electron density into capsule carbohydrates surrounding bacteria, both in tissues (Cagle et al., 1972) and in soils (Fig. 3a; Foster, 1978). Neutral carbohydrates may be stained by lanthanum hydroxide (Revel and Karnovsky, 1967), and La has been used to demonstrate mucilage secreted by roots into soil fabrics (Fig. 2). Carbohydrates which do not react with heavy metals may be induced to do so by prior chemical treatment. For instance, vic-glycol groups in carbohydrates may be oxidized by periodic acid to generate aldehyde groups, to which organic bridging compounds can be added which do complex heavy metals. Examples are the methenamine-silver method (PAMS) of Rambourg (1967), the thiocarbonylhydrazide-Os method (PATO) of Hander et al. (1964) and the thiocarbonyldrazide-silver proteinate method (PATSP) of Thiery (1967).

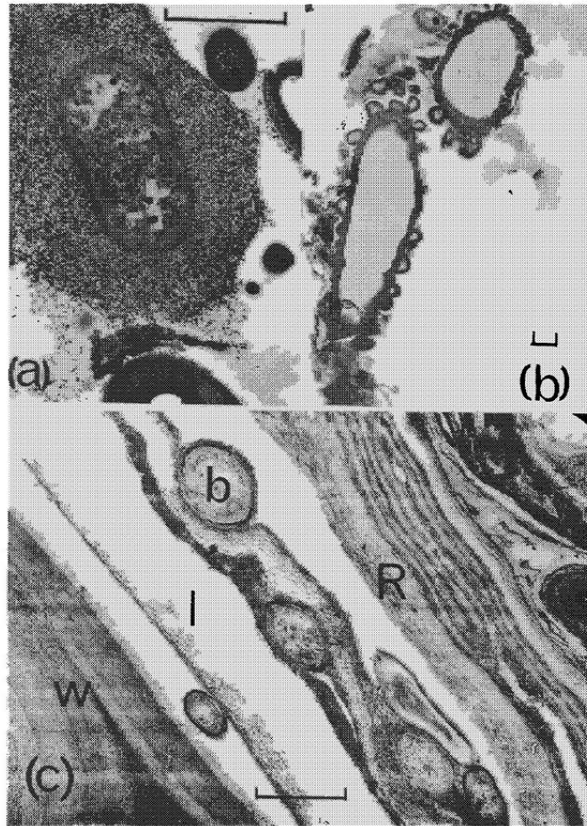


FIG. 3 Examples of soil organics recognized by their position or morphology: (a) fibrous bacterial capsule demonstrated by ruthenium red- OsO_4 ; (b) arthropod appendages Os/Pb; (c) plant cell walls at various stages of decomposition. At I the wall is reduced to a fibrous lignin skeleton and at R to convoluted multilamellate remnants. Os/Pb.

A variety of heavy metal compounds have been used to demonstrate particular biochemicals in biological tissues. These include salts of bismuth (Albersheim and Killias, 1963), indium (Watson and Aldridge, 1961), and vanadium (Callahan and Horner, 1964) for nucleic acids; phosphotungstic acid for proteins, and, at pH 1, for certain carbohydrates (Pearse, 1972), etc. These tests have proved unspecific when applied to soil fabrics, possibly because other soil components have a high affinity for heavy metals. Included are other soil organics such as humic compounds and certain clay minerals (Flaig et al., 1975; Forbes et al., 1976; Zuniro and Martin, 1977; Zimdahl and Hassett, 1977).

Enzymes have been located precisely in biological tissues by making use of their specific catalytic properties. Techniques have been published for a wide range of enzymes, including cellulases, dehydrogenases, esterases, lipases, oxidases, peroxidases, peptidases, phosphatases, phosphorylases, sulfatases, and transferases (Pearse, 1972; Hayat, 1970, 1975). It has been possible to adapt some of these methods for use in soil fabrics (Foster, 1978; Sec. III.D.2). Rigorous controls employing specific enzyme inhibitors or substrate-free media are necessary since all these methods generate heavy-metal-containing products in order that they may be seen in the electron microscope, and heavy metal compounds in the media may become nonspecifically adsorbed onto soil components (Sec. III.D.2). Some enzymes which may be readily demonstrated in roots removed from the soil cannot be demonstrated in intact roots still embedded in the soil fabric. Possible reasons for failure are (1) reaction between constituents of the enzyme-specific media and soil components; (2) soil components may overcome the buffering capacity of experimental media so that the optimal conditions of enzyme action are not obtained; and (3) the enzymes may be denatured by soil polyphenolics transported to the enzyme by the reagents (Lyr, 1961; Ladd and Butler, 1969; Skujins, 1976).

Other soil components may be identified by treating the bulk specimen or ultrathin sections with solvents or materials which modify the reactive sites in the components so that they are no longer stained by the heavy metals. The components are then localized in soil fabrics by the appearance of electron-transparent gaps which were previously electron-dense. For example, sulfur bacteria in soil fabrics may be recognized by the loss of electron density of cellular inclusions when the sections are floated on CS₂. Similarly, acetylation will block the reaction between OsO₄ and polyphenols in plant tissues (Bland et al., 1971). Pure enzymes are now commercially available and may be used to localize particular biochemicals in ultrathin sections with a high degree of specificity. By floating serial sections on a variety of enzymes, different biochemicals can be located in the same fabric.

Organics, which are not visible after conventional fixation may nevertheless be located in situ in soil fabrics by enzyme digestion techniques. For example, a particular class of carbohydrate can be localized by treating

alternate serial sections, first with a specific hydrolytic enzyme and then with a periodic acid-ligand-silver reagent. All the sites in the soil fabric which contain vic-glycol groups become electron-dense in the enzyme-untreated sections, but those sites which contain the enzyme-specific carbohydrate remain clear in the enzyme-treated sections. Identification and localization of any soil organic is therefore limited only by the availability and purity of suitable hydrolytic enzymes.

3. Dehydration, Embedment, and Sample Selection

Methods used to dehydrate soils for electron microscopy include simple heating, air-drying, freeze-drying, critical point drying, and solvent exchange (Smart, 1974). Of these, only the latter three are likely to preserve organic matter in its native state. Solvent exchange at 4°C has proved the most successful for agar-stabilized or clay-rich fabrics. The specimens are then enclosed in plastic (Glauert, 1974). The introduction of very fluid resins (Spurr, 1969) has greatly facilitated the embedment of soil fabrics, but for good embedment some clay soils need soaking in the monomers for a period of weeks and even months. In order to hold hard minerals in place during ultrathin sectioning, and to prevent compression artifacts, it is necessary to use much harder embedments than are commonly employed for biological specimens.

It is usually impossible to cut ultrathin sections of soils with glass knives, and because diamond knives are both fragile and costly, it is necessary to select areas of soil fabrics which are free from sand and silt. The cured plastic blocks may be polished and suitable regions selected by examining the polished surface by reflected light. Alternatively, thick sections may be cut by a sledge microtome and examined in transmitted light. Humified materials are stained brown by OsO_4 , but other organic matter must be stained, for example with toluidine blue, to make it visible. Materials which stain with organic dyes may not be electron-dense, and some materials which do not stain may nevertheless be very electron-dense.

Mineral-free regions of the fabric may be isolated for ultramicrotomy, manually or mechanically (Reid, 1974). The best sections are obtained by cutting very small pyramids (0.1 to 0.5 mm side). Since many of the mineral components of soil fabrics do not cut, even with a diamond knife, but are displaced from the fabric during cutting, sections are often fragile and fragmentary. Observations are therefore restricted to small regions of the fabric with specific interest, and since the best sections are obtained from regions rich in clay or organic matter, observations are biased toward these areas (Sec. IV).

III. IDENTIFICATION OF INDIVIDUAL ORGANIC COMPONENTS

In some cases, organic components of soil can be recognized as being derived from plant, animal, or microbial sources by their position in the soil fabric (e.g., root-derived mucilages, Fig. 2), their characteristic morphology (e.g., invertebrate exoskeletons, Fig. 3b), or their ultrastructure (e.g., fragments of plant cell wall, Figs. 2, and 3c). Other materials may have no distinctive morphological features and are identified by their reaction with particular heavy metal stains. Many amorphous organic materials are of indeterminate origin, especially when they are of submicrometer size.

A. Materials from Plants

Materials derived from plants can be separated into two broad groups—those released into the soil from living tissues and those released from senescent or dead tissues. This distinction may be useful in understanding the localization of organic components in the soil fabric and their susceptibility to decomposition. The first group will contain normal cell metabolites which passively leak out of plant roots (exudates) and extracellular polysaccharides and enzymes which are actively secreted into the surrounding soil. The second group will consist of the soluble products of cell lysis (lysates) released on the death of the cells, cytoplasmic debris within the cells of root, leaf and branch litter (denatured proteins etc.), and eventually the cell walls themselves (see Fig. 4).

1. Root Exudates, Lysates, and Secretions

Most of the material in the root exudates and lysates will be low-molecular-weight, water-soluble compounds which are not fixed in situ. Possibly, during cell lysis some polyphenolic compounds may be formed and will remain at the site of formation. Plant roots, including those from axenic soils, are covered with a layer of mucilage secreted by root cap and epidermal cells. Demonstration of the mucilage requires careful fixation to avoid shrinkage or displacement and special staining, since the mucilage is not directly visible by light microscopy and has only a weak electron density. Mucilage is normally from 1 to 10 μm thick in dry soils, but may reach to 50 μm around grass roots from well-irrigated soils. Since the weight of mucilage secreted by a wheat crop may equal that of the grain harvested, mucilage may be an important source of soil organic matter (Samtsevich, 1965).

Mucilage is composed mainly of carbohydrates (Floyd and Ohrologge, 1970; Oades, 1978) and is detectable as a granular, open-textured material after conventional fixation (Dart and Mercer, 1964). Its slight electron density is probably due to minor constituents such as amino acids and

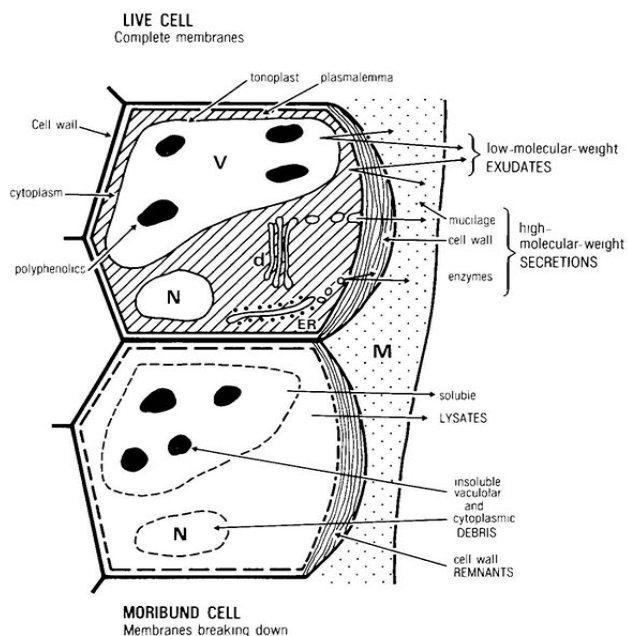


FIG. 4 Diagram of root cells to show the sources of different types of root-derived organic matter in soils.

polyphenolics. Mucilage is best demonstrated by treatment with lanthanum hydroxide (Fig. 2) or by specific periodate-ligand-silver reagents (Pickett-Heaps, 1967). Except where it is protected by clay particles, mucilage is quickly invaded by soil microorganisms (Figs. 2 and 5a) and becomes mixed with bacterial capsule and slime materials as well as colloidal organic and inorganic soil constituents (Fig. 5a). It therefore soon loses its distinctive ultrastructural and biochemical identity, and cannot be unequivocally identified in soil fabrics (Sec. III.D.1).

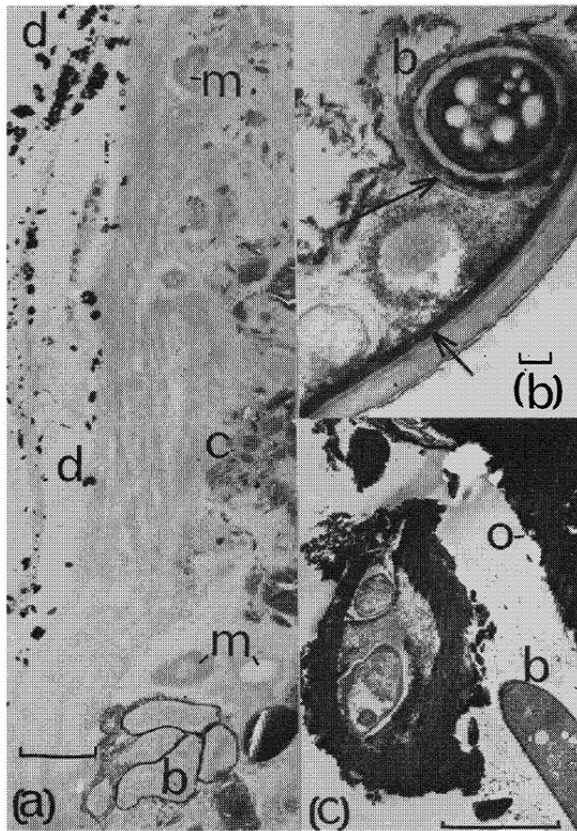


FIG. 5 Localization of enzymes: (a) Acid phosphatase. Certain bacteria b show a positive reaction, whereas adjacent ones m are negative. Remnants of cytoplasmic debris d are also reactive. (b) Peroxidase at the root-soil interface of rice. A positive reaction is obtained in the root cell wall and in the capsule of sulfur bacteria (arrows) Os/Pb. (c) Succinic dehydrogenase. The reaction product is precipitated at the plasma-lemma of soil bacteria, but there is also nonspecific staining of soil organics.

2. Cytoplasmic Debris and Cell Wall Materials

Decomposing root systems and shed aerial parts are a major source of organic matter in soils. There is increasing evidence that the release of organic C into soil from actively growing plant roots has been grossly underestimated and will represent a major input. Growth cabinet experiments employing ^{14}C tracer (Barber and Martin, 1976; Martin, 1977b) have given evidence that the amount of C released from wheat and barley roots between seeding and early tillering may represent up to 10% of the C of the net photosynthate. Other growth cabinet experiments (Sauerbeck and Johnen, 1977) have shown that the mass of wheat roots washed out of the soil at flowering represents only a small fraction of the total root production. The amount of root C converted to CO_2 during root growth is nearly three times the amount recovered at harvest. Data for field crops have supported the growth cabinet experiments. Jenkinson and Rayner (1977) have estimated that organic matter equivalent to $1200 \text{ kg C ha}^{-1}$ enters the top 23 cm of soil in the unmanured plot of the Broadbalk continuous wheat experiment each year. This value compares with the value from other experiments (Welbank et al., 1974) for a dry weight of wheat roots 6 weeks before harvest equivalent to 300 kg C ha^{-1} . These values are in strong contrast with earlier estimates of carbon loss from cereal roots as root exudates equivalent to less than 2% of the plant dry weight (Rovira and Davey, 1974).

Because of their high carbohydrate content, plant cell walls stain intensely with silver after periodate and ligand treatment (Fig. 5a). This heavy staining reaction, combined with the greater thickness of plant cell walls, allows quite small cell wall fragments to be recognized in soil fabrics and to be distinguished from remnants of the cell walls of bacteria (Fig. 5b).

The epidermal and outer cortical cells of the cereal root may be invaded by bacteria while the stele is still functional (Foster and Rovira, 1976, 1978). By anthesis, the outer root cells may be reduced to the polyphenol-rich middle and terminal lamellae of the cell walls. In freshly deposited materials, these layers are the only ones with significant electron density after OsO_4 or KMnO_4 fixation. As the more degradable carbohydrates are utilized by the soil microflora, polyphenolic groups in the lignins are progressively unmasked, so that the cell walls become increasingly electron-dense after conventional fixation (Fig. 3c; Foster 1978). With the removal of most of the carbohydrate, plant cell walls become distorted and compressed and give rise to multilamellate complexes (R, Fig. 3c) similar to those seen in peat (Dart et al., 1969). Most of the cell contents are lysed when the cells die, but some materials such as resins and polyphenols may resist decay. Cell fragments impregnated with these materials may be frequently encountered in fabrics of soils from conifer forests (Foster and Marks, 1967).

B. Materials of Microbial Origin

Soil microorganisms are a significant component of the total soil organic matter and have been estimated to represent 2 to 3% of the organic C in a range of topsoils (Jenkinson and Powlson, 1976). Microbial cells and fungal hyphae are readily seen in soil suspensions using light microscopy; SEM and TEM studies have demonstrated the morphology of soil microorganisms and their spatial distribution on the surfaces of soil particles and roots (Jenny and Grossenbacher, 1963; Dart and Mercer, 1964; Guckert et al., 1975; Foster and Rovira, 1976, 1978).

Many microorganisms produce carbohydrate capsule materials and other organic gels (Webley et al., 1965). These react neither with the fixatives nor with the usual electron-dense stains and thus appear as electron-transparent spaces between the microorganisms and the surrounding clay fabric (Fig. 1). The capsule material of some species stains with ruthenium red- OsO_4 , which has been used to demonstrate an extensive, fibrous glycocalyx surrounding a bacterium from a forest soil (Fig. 3a). The fibrils are about 8 nm in diameter and over 1 μm long. Adjacent organisms are surrounded by material which reacts neither with the ruthenium OsO_4 or the lead stain, but which can be located as an electron-transparent space between these cells and the Ru-Os-reactive capsule of the organisms nearby. In fabrics of soils from under grass, the Ru-Os complex frequently reveals extensive deposits of capsule carbohydrates which persist even after the bacteria themselves have autolyzed (Fig. 6). The soil crumbs may be permeated by Ru-Os-reactive fibrils which, as in Fig. 6, bind the various inorganic and organic components of the soil fabric together. Positive staining of Ru-Os-negative capsule materials may sometimes be achieved by the periodate-ligand-silver method (Kilbertus and Reisinger, 1975).

In contrast to the capsule material, the cell walls of bacteria are stained after conventional fixation (Figs. 2 and 3c), but the walls increase markedly in electron density after treatment with periodate-ligand-silver reagents. PAMS has been used to demonstrate the wall of a bacterium embedded in the mucigel (Fig. 5a). By analogy, remnants of bacterial cell walls can be recognized in fabrics when the organism has died and the wall has fragmented (Fig. 5b).

Bacteria also deposit in their cells organic storage products, such as polyhydroxybutyrate, which appears as electron-translucent material in the cytoplasm (Figs. 1 and 2). Electron-dense granules of polyphosphate (Figs. 2 and 3a), also occur in bacterial cells and may be released into the soil when the cells autolyze.

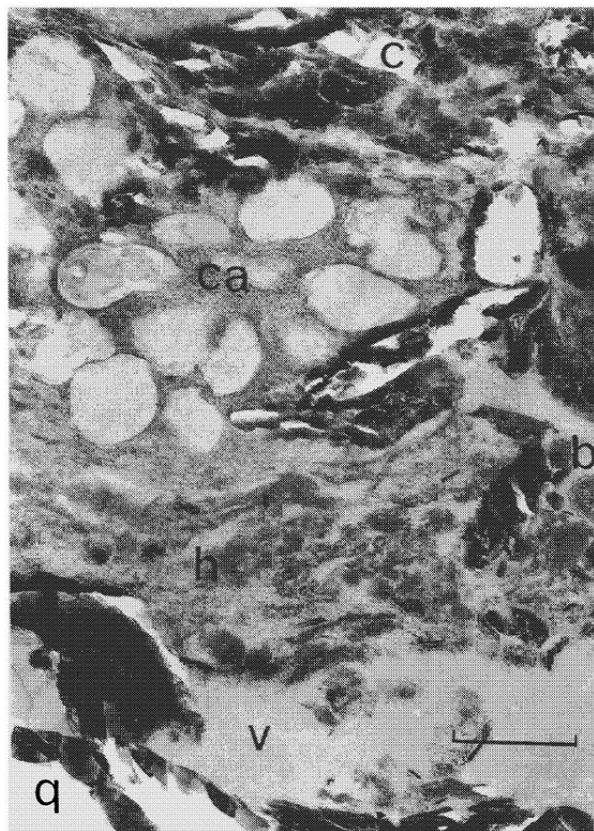


FIG. 6 Remnants of capsule material (ca: demonstrated by ruthenium red- OsO_4) and humic materials which bind mineral components of a soil crumb. Ru/ OsO_4 /Pb.

C. Materials of Animal Origin

Organic materials from animal sources may be grouped into skeletal remains, secretory and excretory products, and artifacts.

1. Skeletal Remains

Most animals escape from soil fabrics during sampling, but some may be quickly immobilized by the organic aldehydes in the fixative, so that occasionally some small invertebrates, such as nematodes, may be encountered. More usually, animal remains consist of hollow appendages (Fig. 3b) and cuticle fragments. These have a characteristic morphology and electron density so that they can be distinguished from the remains of plants. Remains of invertebrates are not uncommon under pastures.

2. Fecal Pellets

Bal (1973) has shown that fecal pellets of soil invertebrates often have a characteristic morphology so that they can be identified and classified in soil fabrics at the light microscope level. In ultrathin sections, fecal pellets are recognized by the enhanced electron density and by the disorganized nature of their contents (f, Fig. 7). The enhanced electron density may be due to proteinaceous materials secreted by the gut of the soil animal, but they might owe their reactivity to polyphenols derived from the surrounding soil. Fecal masses sometimes contain morphologically recognizable plant remains, but these are soon invaded by microorganisms which leave characteristic lysis holes. Extensive bacterial colonies are often found in the soil fabric near fecal deposits (b, Fig. 7). In many soils, plant fragments and other organic matter may be enclosed completely by cutans of clay (Foster, 1978) and these deposits may be physically protected from decay. The recycling of these organic materials may depend on mechanical fragmentation in the guts of soil animals.

3. Artifacts

Worm burrows and some termite tunnels may be recognized at the ultrastructural level by their size and their characteristic ultrastructure. They consist mainly of amorphous electron-transparent materials, but termite tunnels and mound materials often contain clay particles and the characteristic remnants of the middle lamellae of plant cell walls.

D. Materials of Indeterminate Origin

1. Amorphous Organic Residues

In nonsterile soil, it is unrealistic to consider polysaccharides released from plants and microorganisms as distinct, separable entities. The



FIG. 7 Fecal material (f) supporting colonies of microorganisms. The fabric also contains humified organic matter, cell wall remnants, and lipoprotein membranes (m) Os/Pb.

term mucigel, introduced by Jenny and Grossenbacher (1963), has been used as being synonymous with "root mucilage." This was not the intention of Jenny and Grossenbacher, who used the term mucigel for "particulating the root and microbial excretion jelly surrounding the cell wall and extending into the root environment." We recommend that the term mucigel be used in its original sense to define the gellike material surrounding plant roots and extending into the soil matrix. Mucigel is probably predominantly plant polysaccharide which encloses intact microbial cells, other material released from plant and microbial cells, and colloidal mineral and organic matter from the bulk soil. The material produced by axenic roots can be called mucilage, but should not be termed mucigel.

In dry-land soils with good aeration, mucigel may be restricted to the root apex and to isolated pockets between the epidermal cells (Foster and Rovira, 1976; 1978; Bowen and Foster, 1978). Bacteria enzymatically lyse the mucigel, leaving electron-transparent holes (Figs. 2 and 5a). Some mucigel-inhabiting bacteria may be elongated and have diameters of only 0.1 to 0.3 μm . In moist tropical regions and in irrigated pastures of warmer regions, mucigel may provide a protected, energy-rich niche for extracellular, nitrogen-fixing microorganisms (von Bulow and Dobereiner, 1975). Mucigel may also be important in maintaining soil structure, especially under grasslands (Martin, 1971) and under cereal crops where the upper few centimeters of soil are probably completely under the influence of the rhizosphere (Rovira, 1965).

Mucigel is stained by the periodate-ligand-silver techniques (Fig. 3a), provided that the periodate treatment is of short duration. Mucigel then has a characteristic granular appearance, which must be due to the carbohydrate fraction of the gel, since the periodate removes the Os from the section. The use of silver demonstrates more clearly the boundary between the cell wall of the root and the mucigel than does the lanthanum technique (Fig. 2). As the plant cell wall remnants, mucigel deposits, and capsule materials are fragmented by the action of the soil fauna, decay, and chemical weathering, characteristic morphological features are lost. This means that although they can be located in soil fabrics, their origin can only be guessed at on the basis of their ultrastructure or their histochemical properties. For example, fibrous fragments which stain with OsO_4 are likely to be lignin residues similar to those in Fig. 3c. Fragments which only appear after ruthenium-Os treatment are probably remains of bacterial capsule, as in Fig. 6. Fragments of mucigel can be recognized in the absence of roots by their characteristic granularity after conventional fixation (Dart and Mercer, 1964; Foster and Rovira, 1976) and by their reaction with La (Fig. 2) or with the periodic acid-silver reagents (Fig. 5a). Eventually organic materials become so comminuted or chemically altered that although they can be detected, it is impossible to determine their origin. Thus, although certain silver-re-

active fragments can be identified as remnants of bacterial or plant cell walls, others are of indeterminate origin (Fig. 5b).

Humified materials react strongly with OsO_4 and can be recognized as amorphous (h, Fig. 6) or stratified (h, Fig. 7) electron-dense materials. In soils under pastures, fragments of irregular shape about $0.1 \mu\text{m}$ in diameter may be common (Fig. 6) and are similar to those seen in peats by Dart et al., (1969). Visser (1963) has shown that humic acids extracted from peats consist of irregular aggregates, but Chen et al., (1976) demonstrated that the appearance of humic materials isolated from soils depends on the pH and other conditions used in their extraction. Thus, humic acid samples from the same source could appear either granular or fibrous. It is likely, therefore, that the morphology of humic materials in ultrathin sections will be determined by the type, pH, and osmolarity of the buffer, the nature of the fixative, and the concentrations of Ca, Mg, Na, etc. in the medium during fixation (Chen et al., 1976). Bloomfield et al., (1976) have shown that the amounts of heavy metal complexed by humified organic matter is pH-dependent. Copper is fixed onto fulvic acids at pH 3 and onto humic acids at pH 6 (Manskaja et al., 1958). A similar pH dependence has been observed for the uptake of lead (Zimdahl and Hasslett, 1977). It may be possible, therefore, to identify different kinds of humic materials by appropriate adjustment of the pH at fixation.

Soils also contain inorganic amorphous materials which may be difficult to distinguish from organic matter. EPM may be used to identify inorganic deposits by determining the ratios of particular elements in them. However, in the absence of EPM, use may be made of the fact that osmium black is removed from ultrathin sections by H_2O_2 . Thus, amorphous organic materials lose their electron density after treatment, but inorganic materials are unaffected. Mixed inorganic/organic colloids may also occur in soils, and these can be identified by suitable combinations of destaining and staining procedures.

2. Soil Enzymes

The precise location of enzymes can be determined to within 10 nm, using the appropriate histochemical methods. It should be possible, therefore, to determine how much of a particular soil enzyme is located within living tissues of plants, animals, and microorganisms and how much is adsorbed onto nonliving organic and inorganic soil constituents.

Both roots and microorganisms produce enzymes, some of which are extracellular and some of which are released into the soil on the death of the cells (Kuprevich and Shcherbakova, 1971; Ladd and Butler, 1975; Skujins, 1976). Enzymes mediating electron transfer are thought to be associated mainly with living cells, and therefore can be used to test the specificity of histochemical techniques. Enzymes active against high-molecular-weight materials may lie free in the soil (Skujins, 1976) or be

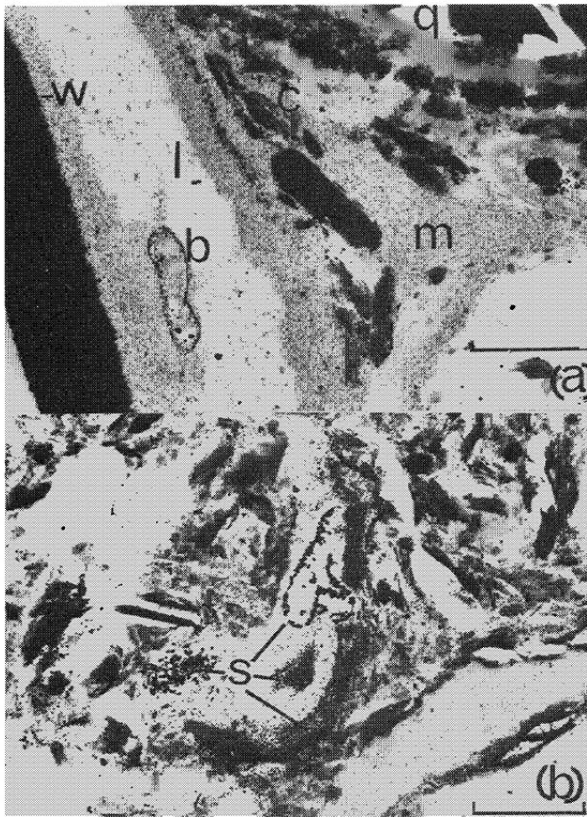


FIG. 8 Localization of vic-glycol groups by the periodic acid-methenamine-silver method. (a) Root-soil interface. There is an intense reaction in the cell walls of the root and bacteria. The mucigel is less reactive (l: lysis of mucigel by bacteria). (b) A region remote from the root. Intense reaction obtained in wall remnants of root and bacteria, but also at other sites where there is no morphologically recognizable material. Ag only.

associated with organic debris or even clay minerals. Tests for acid phosphatase have located the enzyme both in the cytoplasmic remnants associated with the cell walls of roots (d, Fig. 8a) and at the surface of the bacterial cells in the rhizosphere and in the general soil fabric. Cell fragments of submicrometer size retain acid phosphatase activity. Fecal materials are often rich in enzymes, and it is possible that soil components may become secondarily invested with enzymes as they pass through the guts of soil animals.

Peroxidase is common in soils, and can be located in the middle lamellae of cell wall fragments by the diaminobenzidine method (Graham and Karnovsky, 1966). Catalase has been located in soil fabrics near rice roots (Fig. 8b), where it is involved in the breakdown of H_2O_2 produced during the oxidation of H_2S to elemental S by rhizosphere microorganisms (Joshi and Hollis, 1977). The enzyme occurs both in the cell walls of the epidermal cells of the rice and in the capsules of the S bacteria (arrows, Fig. 8b).

Techniques using heavy metals to locate enzymes in soil fabrics must be interpreted with caution. Tests for succinic dehydrogenase demonstrated the enzyme at the plasma membrane of bacterial cells (Fig. 8c), but adjacent soil organic matter is also electron-dense. Since this enzyme is usually associated only with living cells, the electron density in the organics must be due to nonspecific adsorption of the metals used in the test. These unspecific reactions limit the usefulness of enzyme cytochemistry in soil fabrics, and at present enzymes have only been unequivocally located in morphologically recognizable cells or cell fragments.

3. Lipoprotein Membranes

Fragments of lipoprotein membranes are common in soil fabrics, both near roots (Foster and Rovira, 1978) and in the bulk soil (m, Fig. 7). Membranes are present in all cellular organisms, but in soil fabrics they are most often associated with colonies of bacteria. Many enzymes are associated with membranes, and since the membrane fragments tend to roll inward, these membranes may constitute one type of protected site where enzymes could persist in soils.

IV. QUANTIFICATION OF COMPONENTS IN SOIL FABRICS

Because it is possible to recognize in electron micrographs the various components of soil fabrics on the basis of their morphology, texture, and histochemical reactions, it is also possible to determine the approximate proportions of each class of component in the soil.

TABLE 4 Relative Volumes of Soil Constituents in Different Soil Fabrics Based on Electron Micrographs

Soil	Vol %									
	Voids	Minerals		Organic Matter						
				Carbohydrates			Polyphenolics			
		Quartz	Clay	Cells	Capsule	Mucilage	Wall ^a	Humus	Wall ^b	
Bulk Soil										
Black earth	20	2	68	2	1	0	0	5	2	
Calcareous red loam	12	4	56	2	0	0	12	14	1	
Rhizosphere										
Calcareous red loam	4	4	27	26	30	0	4	1	2	
Wet land soil	4	10	33	2	2	35	10	4	1	

^aElectron-transparent and after OsO₄ fixation.

^bElectron-dense after OsO₄ fixation.

Smart (1966), Foster and Evans (1971), and Tovey et al., (1974) have described some of the methods in quantitative analysis of soil fabrics. Automatic systems which operate on electron micrographs and even on the output of scanning electron microscopes are commercially available. Table 4 shows the results of a crude qualitative analysis for fragments of four Australian soils.

Electron micrographs of the soil fabrics were sampled at 200 points arranged in a regular grid, and the nature of the material at each point was recorded. Minerals were differentiated into clay or quartz on the basis of internal morphology or fragmentation characteristics. Organic matter was differentiated into three main classes: (1) living cells such as microorganisms; (2) electron-transparent materials, assumed to be carbohydrate-rich; and (3) electron-dense, polyphenol-rich materials. Further differentiation was made on the basis of ultrastructure (texture) to give six classes. These are (1) bacterial cells; (2) bacterial capsules; (3) mucigel; (4) carbohydrate-rich cell wall layers; (5) polyphenol-rich cell wall layers; and (6) polyphenol-rich, amorphous humic materials.

The number of points (out of 200) falling on each category of soil component (including voids) was determined for each class of compound (vol % of the fabric). Several areas were counted for each type of specimen and results were expressed as the mean for each soil type. There were consistent differences between the various types of specimen, despite high standard deviation of means due both to the natural heterogeneity of the soils at the ultrastructural level and to the small volume of soil represented in each electron micrograph. The proportion of quartz calculated for the soils would be lower than that obtained by mechanical analysis because of the necessity to avoid regions rich in hard minerals. However, from the point of view of the soil microbiologist, these results may be more meaningful than gross analyses, because they describe the immediate environment of particular soil microorganisms.

The results in Table 4 emphasize the differences in the microbial environments in bulk soils and in rhizospheres. Void space was significantly smaller in the rhizosphere soils, because mechanical pressures from growing roots compact the soil around them (Blevins et al., 1970) and because most of the available void is filled with gels. These results indicate that quantification can be attempted using ultrathin sections. Unfortunately, in order that the various components may be unambiguously identified and that a reasonable number of sampling points may fall on each class of material, large (10,000x) magnifications are necessary. Thus the volume of material sampled in each electron micrograph is very small ($\sim 5 \times 10^{-12} \text{ cm}^3$). Using conventional TEM, the number of areas of fabric of a size similar to that shown in Fig. 1 and which survive the cutting and staining procedures is very small ($\sim 0.1\%$ of sections examined). The use of STEM may increase the survival rate of large areas of soil fabrics, since thicker sections can be used.

V. FUTURE DEVELOPMENTS

Although *in situ* studies using electron optical techniques have not yet explained the stability of polysaccharide or nitrogenous components of soil fabrics, some insights into possible causes are being provided. For example, Figs. 1 and 2 show that both individual bacteria and colonies may be enveloped in clay or may be separated from recognizable organic matter by tens of micrometers. Figure 8b shows a soil fabric in which there are present various types of silver-reactive carbohydrate, but no microbial cells. Such studies could demonstrate organic matter which is in voids too small for access to soil microflora and that which is blanketed by clays or impregnated with polyphenolics. These observations could be extended by the use of autoradiography of ^{14}C -labeled materials at the ultrastructural level, using liquid emulsions, or by the use of antibodies to which electron-dense tags have been added (Pearse, 1972). Since antibody labeling can be performed on ultrathin sections, different biochemicals could be localized in successive sections of the same soil fabric. EPM could show if carbohydrate were impregnated with Cu, Zn, or other metals which would inhibit its decomposition (Martin et al., 1966).

Many of the uncertainties on soil enzymes will be removed using the techniques described in Secs. II.B.2.c and II.D.2. Provided that unspecific reactions with soil organic matter are not excessive, it will be possible, after examining a number of sections, to determine the relative proportions of an enzymic activity associated with whole cells or with cell fragments or sorbed onto clays or humic materials. Alternatively, by combination of EPM with STEM on thicker sections, it may be possible to locate organic products of enzyme reactions, e.g., P-containing products, and so avoid artifacts due to nonspecific adsorption of heavy metals.

The electron-optical techniques can also lead to a better understanding of the dynamics of soil biomass and energy flow through soils. Several authors (Clark and Paul, 1970; Gray and Williams, 1971; Jenkinson and Ladd, this volume, Chap. 10) have compared estimates of total microbial numbers in soil, obtained by direct microscopy, with estimates of the amount of available C, and have concluded that cell division is limited to 2 to 3 times per year. Yet the electron micrographs published here (Fig. 2) and elsewhere (Foster and Rovira, 1976, 1978) have shown colonies of bacteria at the soil-root interface and within pockets of organic matter in the bulk soil, with individual cells which contain large amounts of inclusion material, indicative of an abundant supply of locally available substrate. It is evident that many of the microorganisms detected by direct microscopy either remain dormant for long periods or are nonviable, even though they may retain osmotic barriers intact (Postgate, 1973). TEM studies of rhizosphere soils support both possibilities. Tests for acid phosphatase in rhizosphere soils showed that only 25% of the recognizable microbial cells gave positive reactions, although their cytoplasmic organ-

ization appeared as complete as that of their unreactive near neighbors (Fig. 8a). Alternatively, soil microflora such as the lobed forms observed in rhizosphere soils but not in the bulk soil (Old and Nicholson, 1975; Foster and Rovira, 1978) may multiply only at root surfaces and may remain dormant at other times.

TEM studies of rhizosphere soil suggest that the microbial populations which result from the release of organic material from the roots occur in small discrete pockets of intense activity, rather than evenly distributed through the soil (Fig. 2). Factors contributing to the discontinuity are (1) the very small proportion of the soil volume occupied by plant roots—usually less than 5% (Wiersum, 1961); and (2) a variation with time in the amount and nature of the organic material released from plant roots (Martin, 1978). It also seems likely that variation in plant-available water will give rise to localized sites at which there is an increased release of root C into the soil (Martin, 1977a). These considerations emphasize the necessity for studies of rhizosphere phenomena to be based on in situ examination of soil in which there is vigorous root growth.

A major limitation to in situ soil studies using TEM of ultrathin sections, is the very small sample size (and large magnifications) necessary to distinguish the various types of colloidal organic matter. Thus, TEM estimates for the different classes of organic matter should be complemented by chemical characterization of material released from the growth and decomposition of plant roots. The plants should be grown in soils retaining their natural structure, and the plant tops supplied with radioisotope (cf. Warembourg and Paul, 1973; Warembourg, 1975). It would then be possible to compare the total amount of radioisotope present in the different fractions of organic matter (chemical or physical) with the presence of the radioisotope in structural components of the soil fabric, as indicated by SEM or TEM. Short-term experiments could provide information on processes involved in the loss of C from actively growing roots, while studies made 6 to 24 months after labeling the plants could identify the chemical nature of the resistant organic matter and its relationship to various components of the soil fabric.

This approach could also be used in nonrhizosphere soil to extend studies such as those by Grossbard on the decomposition of litter (Grossbard, 1971) and herbicides (Grossbard, 1973), and would be applicable to environmental studies on the movement of toxic chemicals or nuclear radioisotopes through different ecosystems.

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REFERENCES

- Albersheim, P., and Killias, U. (1963). The use of bismuth as an electron stain for nucleic acids. *J. Cell Biol.* **17**: 93-103.
- Babel, U. (1971). Methods of investigating the micromorphology of humus. In *Ecological Studies Analysis and Synthesis* (H. Ellenberg, Ed.), Vol. 2. Springer, Berlin, pp. 164-168.
- Babel, U. (1975). Micromorphology of soil organic matter. In *Soil Components* (J. E. Gieseking, Ed.), Vol. 1. Springer, Berlin, pp. 369-473.
- Bahr, G. F. (1954). Osmium tetroxide and ruthenium tetroxide and their reactions with biologically important substances: Electron stains, III. *Exp. Cell Res.* **7**: 457-479.
- Bal, L. (1973). *Micromorphological Analysis of Soils*. Soil Survey Institute, Wageningen, The Netherlands.
- Barber, D. A., and Martin, J. K. (1976). The release of organic substances by cereal roots into soils. *New Phytol.* **76**: 69-80.
- Bland, D. E., Foster, R. C., and Logan, A. F. (1971). The mechanism of permanganate and osmium tetroxide fixation and the distribution of lignin in the cell wall of *Pinus radiata*. *Holzforschung* **25**: 137-143.
- Blevins, R. L., Holowaychuk, N., and Wilding, L. P. (1970). Micromorphology of soil fabric at free root-soil interface. *Soil Sci. Soc. Amer. Proc.* **34**: 460-465.
- Bloomfield, C., Kelso, W. I., and Pruder, G. (1976). Reactions between metals and humified organic matter. *J. Soil Sci.* **27**: 16-31.
- Bowen, G. D., and Foster, R. C. (1978). Dynamics of microbial colonization of plant roots. In *Soil Microbiology and Plant Nutrition* (W. J. Broughton and C. K. John, Eds.). University of Malaya Press, Kuala Lumpur, pp. 14-31.
- Bremner, J. M. (1967). Nitrogenous compounds. In *Soil Biochemistry* (A. D. McLaren and G. H. Peterson, Eds.), Vol. 1. Marcel Dekker, New York, pp. 19-66.
- Brewer, R. (1964). *Fabric and Mineral Analysis of Soils*, Wiley, New York.
- Bullock, P. (1974). The micromorphology of soil organic matter: A synthesis of recent research. In *Soil Microscopy* (G. K. Rutherford, Ed.). Limestone Press, Kingston, Ontario, pp. 49-66.
- Bulow, J. F. W. von, and Dobereiner, J. (1975). Potential for nitrogen fixation in maize genotypes in Brazil. *Proc. Nat. Acad. Sci. U.S.* **72**: 2389-2393.

- Cagle, C. D., Pfister, R. M., and Vela, G. R. (1972). Improved staining of extracellular polymers for electron microscopy. *Appl. Microbiol.* 24: 477-487.
- Callahan, W. P., and Horner, J. A. (1964). The use of vanadium as a stain for electron microscopy. *J. Cell Biol.* 20: 350-356.
- Chen, Y., Banin, A., and Schnitzer, M. (1976). Use of the SEM for structural studies of soil and soil components. In Proc. 9th. Annual SEM Symposium, Part III. Chicago 177 Research Institute, Chicago, pp. 425-432.
- Cheshire, M. V. (1977). Origins and stability of polysaccharide. *J. Soil Sci.* 28: 1-10.
- Clark, F. E., and Paul, E. A. (1970). The microflora of grassland. *Advan. Agron.* 22: 375-435.
- Clowes, F. A. L., and Juniper, B. E. (1968). *Plant Cells*. Blackwell, Oxford.
- Dart, P. J., and Mercer, F. V. (1964). The legume rhizosphere. *Arch. Microbiol.* 47: 344-378.
- Dart, P. J., Roughley, R. J., and Chandler, M. R. (1969). Peat culture of *Rhizobium trifolii*: An examination by electron microscopy. *J. Appl. Bacteriol.* 32: 352-357.
- Davies, R. I., Coulson, C. B., and Lewis, D. A. (1964). Polyphenols in plant, humus, and soil, III: Stabilization of gelatin by polyphenol tannin. *J. Soil Sci.* 15: 229-309.
- Flaig, W., Beutelspacher, H., and Rietz, E. (1975). Chemical composition and physical properties of humic substances. In *Soil Components* (J. E. Gieseking, Ed.), Vol. 1. Springer, Berlin, pp. 1-211.
- Floyd, R. A., and Ohrologge, A. J. (1970). Gel formation on nodal root-surfaces of *Zea mays*, I. *Plant Soil* 33: 331-343.
- Forbes, E. A., Posner, A. M., and Quirk, J. P. (1976). The specific adsorption of divalent Cd, Co, Cu, Pb, Zn on goethite. *J. Soil Sci.* 27: 154-166.
- Foster, R. C. (1978). Ultramicro-morphology of some South Australian Soils. In *Modification of Soil Structure* (W. W. Emerson, R. D. Bond, and A. R. Dexter, Eds.). Wiley, London, pp. 103-109.
- Foster, R. C., and Marks, G. C. (1967). Observation on the mycorrhizas of forest trees, II: The rhizosphere of *Pinus radiata* D. Don. *Aust. J. Biol. Sci.* 20, 915-26.
- Foster, R. C., and Rovira, A. D. (1976). Ultrastructure of the wheat rhizosphere. *New Phytol.* 76: 343-352.

- Foster, R. C., and Rovira, A. D. (1978). The ultrastructure of the rhizosphere of *Trifolium subterraneum* L. in Microbial Ecology (M. W. Loutit and J. A. R. Miles, Eds.). Springer, Berlin, pp. 278-290.
- Foster, R. H., and Evans, J. S. (1971). Image analysis of clay fabric by Quantimet. The Microscope 19: 377-401.
- Glauert, A. M. (1974). Fixation, dehydration, and embedding of biological specimens. In Practical Methods in Electron Microscopy (A. M. Glauert, Ed.). Vol. 3. North Holland, Amsterdam, pp. 1-207.
- Graham, R. C., and Karnovsky, M. (1966). The early stages of absorption of injected horse radish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 14: 291-302.
- Gray, T. R. G., and Williams, S. T. (1971). Microbial productivity in soil. In Microbes and Biological Productivity (Symposia of the Society for General Microbiology, 21) (D. E. Hughes and A. H. Rose, Eds.). Cambridge University Press, Cambridge, pp. 255-286.
- Grossbard, E. (1971). The utilization and translocation by microorganisms of carbon-14 derived from the decomposition of plant residues in soil. J. Gen. Microbiol. 66: 339-348.
- Grossbard, E. (1973). An autoradiographic technique to observe uptake and utilization by microorganisms of carbon-14 derived from labelled herbicides in soil. Bull. Ecol. Res. Comm., Stockholm 17: 275-276.
- Guckert, A., Breisch, H., and Reisinger, O. (1975). Interface sol-racine, 1: Étude au microscope électronique des relations mucigel-argile-microorganismes. Soil Biol. Biochem. 7: 241-250.
- Hake, T. (1965). Studies on the reactions of OsO₄ and KMnO₄ with amino acids, peptides, and proteins. In Quantitative Electron Microscopy (G. F. Bahr and E. H. Zeitler, Eds.). Williams & Wilkins, Baltimore, pp. 470-474.
- Hall, C. E., Jakus, M. E., and Schmitt, F. O. (1945). Structure of certain muscle fibrils as revealed by electron stains. J. Appl. Physiol. 16: 459-465.
- Hanker, J. S., Seaman, A. R., Weiss, L. P., Ueno, H., Bergman, R. A. L., and Seligman, A. M. (1964). Osmiophilic Reagents: New cytochemical principle for light and electron microscopy. Science 146: 1039-1043.
- Hawker, L. E., Linton, A. H., Folkes, B. F., and Carlile, M. J. (1962). An Introduction to the Biology of Microorganisms. Edward Arnold, London, p. 272.

- Hayat, M. A. (1970). Principles and Techniques of Electron Microscopy: Biological Applications, Vol. 1, Van Nostrand Reinhold, New York.
- Hayat, M. A. (1975). Positive Staining for Electron Microscopy: Van Nostrand Reinhold, New York.
- Jenkinson, D. S., and Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil, V: A method for measuring soil biomass. *Soil Biol. Biochem.* 8: 209-213.
- Jenkinson, D. S., and Rayner, J. H. (1977). The turnover of soil organic material in some of the Rothamsted classical experiments. *Soil Sci.* 123: 298-305.
- Jenny, H., and Grossenbacher, K. A. (1963). Root-soil boundary zones as seen in the electron microscope. *Soil Sci. Soc. Amer. Proc.* 27: 273-277.
- Joshi, M. M., and Hollis, J. P. (1977). Interaction of *Beggiatoa* and rice plant: Detoxification of hydrogen sulphide in the rice rhizosphere. *Science* 195: 179-180.
- Juniper, B. E., Cox, G. C., Gilchrist, A. J., and Williams, P. R. (1970). Techniques for Plant Electron Microscopy. Blackwell, Oxford.
- Kay, D. H. (1967). Techniques for Electron Microscopy. Blackwell, Oxford.
- Kilbertus, G., and Reisinger, O. (1975). Degradation du materiel vegetal activit  in vitro et in situ de quelques microorganismes. *Rev. Ecol. Biol. Sol* 12: 363-374.
- Kuprevich, V. F., and Shcherbakova, T. A. (1971). Comparative enzymatic activity in diverse types of soil. In *Soil Biochemistry* (A. D. McLaren and J. Skujin s, Eds.), Vol. 2. Marcel Dekker, New York, pp. 167-201.
- Ladd, J. N., and Butler, J. H. A. (1969). The effect of methylation of humic acids on their influence on proteolytic enzyme activity. *Aust. J. Soil Res.* 7: 263-268.
- Ladd, J. N., and Butler, J. H. A. (1971). Inhibition by soil humic acids of native and acetylated proteolytic enzymes. *Soil Biol. Biochem.* 3: 157-160.
- Ladd, J. N., and Butler, J. H. A. (1975). Humus-enzyme systems and synthetic organic polymer-enzyme analogs. In *Soil Biochemistry* (E. A. Paul and A. D. McLaren, Eds.), Vol. 4. Marcel Dekker, New York, pp. 143-194.
- Luft, J. H. (1971). Ruthenium red and violet: Chemistry, purification, and methods for electron microscopy and mechanism of action. *Anat. Rec.* 171: 347-368.

- Lyr, H. (1961). Hemmungsanalytische Untersuchungen an einigen Ektoenzymen holzerstorender Pilze. *Enzymologia* 23: 231-248.
- McKyes, E., Sethi, A., and Young, R. N. (1974). Amorphous coatings on particles of sensitive clay soils. *Clays Clay Minerals* 22: 427-433.
- Manskaja, S. M., Drozdova, T. M., and Emel'Yanova, M. P. (1958). Copper fixation by various forms of natural-organic compounds. *Sov. Soil Sci.* 6: 623-629.
- Martin, J. K. (1977a). Effect of soil moisture on the release of organic carbon from wheat roots. *Soil Biol. Biochem.* 9: 303-304.
- Martin, J. K. (1977b). Factors influencing the loss of organic carbon from wheat roots. *Soil Biol. Biochem.* 9: 1-7.
- Martin, J. K. (1978). The variation with plant age of root carbon available to soil microflora. In *Microbial Ecology* (M. W. Loutit and J. A. R. Miles, Eds.). Springer, Berlin, pp. 299-302.
- Martin, J. P. (1971). Decomposition and binding action of polysaccharides in soil. *Soil Biol. Biochem.* 3: 33-41.
- Martin, J. P., Erwin, J. O., and Shepherd, R. A. (1966). Decomposition of iron, aluminium, zinc, and copper salts or complexes of some microbial and plant polysaccharides in soil. *Soil Sci. Soc. Amer. Proc.* 30: 196-200.
- Meek, G. A. (1970). *Practical Electron Microscopy for Biologists*. Wiley-Interscience, London.
- Mercer, E. H., and Birbeck, M. S. C. (1972). *Electron Microscopy: A Handbook for Biologists*. Blackwell, Oxford.
- Oades, J. M. (1978). Mucilages at the root surface. *J. Soil Sci.* 29: 1-16.
- Old, K. M., and Nicholson, T. H. (1975). Electron microscopical studies of the microflora of roots of sand dune grasses. *New Phytol.* 74: 51-58.
- Pearse, A. G. E. (1972). *Histochemistry: Theoretical and Applied*. Churchill, Edinburgh and London.
- Pease, D. C. (1964). *Histological Techniques for Electron Microscopy*. Academic Press, London and New York.
- Pease, D. C. (1975). Phosphotungstic acid as a specific electron stain for complex carbohydrates. *J. Histochem. Cytochem.* 18: 445-458.
- Pickett-Heaps, J. D. (1967). Preliminary attempts at ultrastructural polysaccharide localization in root-tip cells. *J. Histochem. Cytochem.* 15: 442-455.

- Postgate, J. R. (1973). The viability of very slow-growing populations: A model for the natural ecosystem. In *Modern Methods in the Study of Microbial Ecology* (T. Rosswall, Ed.). Ecological Research Committee/NFR, Stockholm, pp. 287-292.
- Rambourg, A. (1967). An improved silver methenamine technique for the detection of periodic acid reactive complex carbohydrates with electron-microscopy. *J. Histochem. Cytochem.* 15: 409-412.
- Reid, N. (1974). Ultramicrotomy. In *Practical Methods in Electron Microscopy* (A. M. Glauert, Ed.), Vol. 3. North Holland, Amsterdam, pp. 213-338.
- Revel, J. P., and Karnovsky, M. J. (1967). Hexagonal array of subunits in intercellular junctions of mouse heart and liver. *J. Cell Biol.* 20: 313-332.
- Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell. Biol.* 17: 208-212.
- Roland J. C., and Vian B. (1970). Localization and ultrastructural characterization of acid polysaccharides in plant cells. *C. R. Acad. Sci., Paris* 271: 572-575.
- Rovira, A. D. (1965). Plant root exudates and their influence upon soil microorganisms. In *Ecology of Soil-borne Plant Pathogens* (K. F. Baker and W. C. Snyder, Eds.). Murray, London, pp. 170-184.
- Rovira, A. D., and Davey, C. B. (1974). Biology of the rhizosphere. In *The Plant Root and its Environment* (E. W. Carson, Ed.). University Press of Virginia, Charlottesville, pp. 153-204.
- Samtsevich, S. A. (1965). Active secretions of plant roots and their significance. *Sov. Plant Physiol.* 12: 731-740.
- Sauerbeck, D. R., and Jochen, B. G. (1977). Root formation and decomposition during plant growth. In *Soil Organic Matter Studies*. (Proc. Symp. 1976), Vol. 1. IAEA, Vienna, pp. 144-148.
- Schlesinger, W. H. (1977). Carbon balance in terrestrial detritus. *Am. Rev. Ecol. Syst.* 8: 51-81.
- Skujinš, J. (1976). Extracellular enzymes in soil. *Crit. Rev. Microbiol.* 4: 383-422.
- Smart, P. (1966). Particle arrangements in kaolin. *Clays Clay Minerals* 15: 241-254.
- Smart, P. (1974). Electron microscope methods in soil micromorphology. In *Soil Microscopy* (G. K. Rutherford, Ed.). Limestone Press, Kingston, Ontario, pp. 190-206.

- Spurr, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26: 31-43.
- Thiéry, J. P. (1967). Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.*, Paris 6: 987-1018.
- Tovey, N. K. (1974). Some applications of electron microscopy to soil engineering. In *Soil Microscopy* (G. K. Rutherford, Ed.). Limestone Press, Kingston, Ontario, pp. 119-142.
- Tovey, N. K., and Kwong, K. Y. (1974). Some aspects of quantitative measurement from electron micrographs of soil structures. In *Soil Microscopy* (G. K. Rutherford, Ed.). Limestone Press, Kingston, Ontario, pp. 207-222.
- Visser, S. (1963). Electron microscopic and electron diffraction patterns of humic acids. *Soil Sci.* 96: 353-356.
- Warembourg, F. R. (1975). Application de techniques radioisotopiques à l'étude de l'activité biologique dans la rhizosphère des plantes. *Rev. Ecol. Biol. Sol* 12: 261-272.
- Warembourg, F. R., and Paul, E. A. (1973). The use of $C^{14}O_2$ canopy techniques for measuring carbon transfer through the plant-soil system. *Plant Soil* 38: 331-345.
- Watson, M. L. (1958). Staining of tissue for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4: 475-484.
- Watson, M. L., and Aldridge, W. G. (1961). Methods for the use of indium as an electron stain for nucleic acids. *J. Biophys. Biochem. Cytol.* 11: 257-272.
- Webley, D. M., Duff, R. B., Bacon, J. S. D., and Farmer, V. C. (1965). A study of polysaccharide-producing organisms occurring in the root region of certain pasture grasses. *J. Soil Sci.* 16: 149-157.
- Welbank, P. J., Gibb, M. J., Taylor, P. J., and Williams, E. D. (1974). Root growth of cereal crops. Rothamsted Exp. Stn. Rep. Part 2, 26-66.
- Wiersum, L. K. (1961). Utilization of soil by the plant root system. *Plant Soil* 15: 189-192.
- Zimdahl, R. L., and Hassett, J. J. (1977). Lead in soil. In *Lead in the Environment* (W. R. Boggess, Ed.). National Science Foundation, Washington, D.C., pp. 93-96.
- Zunero, H., and Martin, J. P. (1977). Metal binding-organic macro-molecules in soil, I. *Soil Sci.* 123: 65-75.